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PRINCIPAL INVESTIGATOR: Wayne D. Lancaster, Ph.D.

CONTRACTING ORGANIZATION: Wayne State University
Detroit, Michigan 48201

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Wayne D. Lancaster, Ph.D.

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
Wayne State University
Detroit, Michigan 48201

E-Mail: wayne@genetics.wayne.edu

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**13. Abstract** ***(Maximum 200 Words)*** *(abstract should contain no proprietary or confidential information)*

The objectives of this study are to (1) analyze gene expression in a model of human ovarian carcinogenesis from a benign to a malignant phenotype in organotypic culture (months 1-18) and (2) to confirm gene expression patterns by RNA analysis (months 19-36). Sufficient RNA for microarray analysis has been isolated and purified. Prior to undertaking the analysis of this RNA we analyzed the reproducibility of the cDNA microarrays originally used to generate preliminary data. We analyzed a single preparation of RNA on a single array in duplicate. The results indicated a significant lack of reproducibility in that about 25% of the cDNAs showed a deviation between the two hybridization reactions >1.5-fold difference. Due to this lack of reproducibility and the fact that the original arrays will not allow rigorous statistical analysis we have decided to utilize oligonucleotide arrays. The advantage is quality control and reproducibility. These experiments will be completed by month 18 of the project. These experiments will consist of hybridizing cRNA from three separate cultures at three stages of progression to the malignant phenotype to Affymetrix gene chips. All experiments being done in triplicate will ensure reliable data to analyze in the second half of the project period.

**14. SUBJECT TERMS**
organotypic (raft) culture of human ovarian surface epithelial CDNA, microarray analysis of gene expression

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**Introduction:** Most epithelial cell cancers (cervix, colon, skin, prostate, breast, etc.) develop from precursor lesions resulting from an accumulation of mutations in growth regulatory genes. Such precursor lesions have not been identified for OVCA but it has been proposed that OVCAs arise by a multistep process through increasingly aggressive stages. We have shown that immortalized human ovarian surface epithelial (HOSE) cells undergo stepwise progression to the malignant phenotype in vitro. We now hypothesize that this phenotypic presentation reflects changes in the expression of genes in biochemical pathways required for transition from benign cells to malignantly transformed cells. The long-range goal of these studies is to identify aberrantly expressed genes in HOSE cells at various stages along the path to the malignant phenotype for the purpose of characterizing biochemical pathways whose expression is dysregulated.

**Body:** To establish the validity of our original choice of microarray product (Invitrogen; Research Genetics) to be used in this project, we tested a single array with two hybridizations with the identical cDNAs. All things being equal, the arrays should yield very similar ratios between any give spot on the array. What we found, however, was that the degree of reproducibility of the Research genetics array was not suitable for our studies. As shown in Figure 1 we compared the hybridization intensities of cDNA made from RNA isolated from an ovarian cancer cell line grown in monolayer culture to itself on the same microarray. The line labeled 1:1 passes through the intercept and if this were a perfect array all dots would lie on the line. The lines labeled 2:1 and 1:2 are two-fold over and under expressed, respectively. Embedded within the array are a series of total genome DNA sequences that are used to align the array as well as for normalization. Figure 2 shows the array hybridization signals for only the total genome sequences within the array.

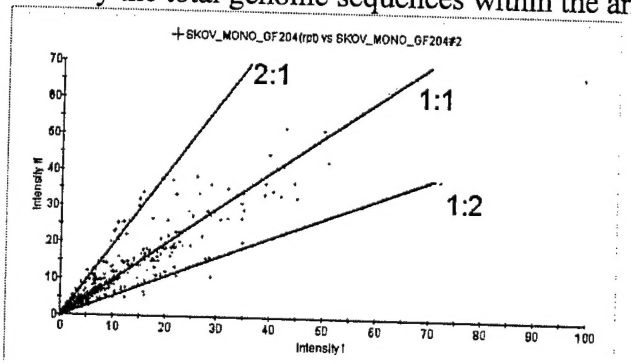


Figure 1. Comparison of signal intensity for all data points for a single cDNA hybridized to the same microarray. The line labeled 1:1 represents equal signal intensity for both hybridizations. Lines labeled 2:1 and 1:2 represent 2-fold over and under expression. In a prefect analysis all of the data points should reside on the 1:1 line.

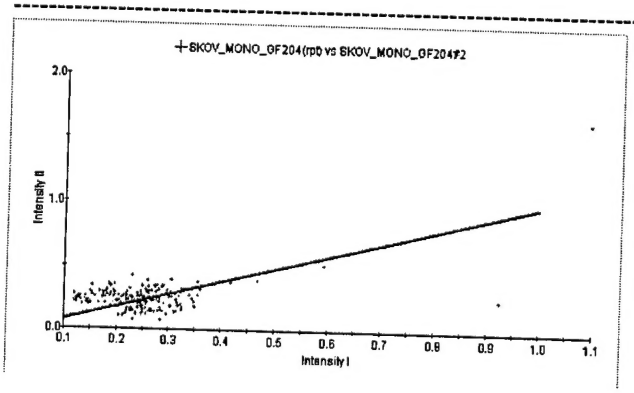


Figure 2. Comparison of signal intensity for only total genomic DNA data points for a single cDNA hybridized to the same microarray. The line within the graph represents unity and in a perfect array all data points should fall on this line.

Although they tend to cluster around unity, there is great variation in signal intensity. If the array is normalized to these data points the variation in signal intensity for any given data point could be quite misleading. The arrays were stripped according to the vendor's instructions and they were checked by

autoradiography using a phosphorimager to ensure all residual signal had been removed. This study clearly shows that the cDNA microarray marketed by Invitrogen will not be of sufficient precision to warrant their use. Another problem with these arrays is that they can be used only 5 times. To ensure that we have sufficient statistical power to properly analyze our data, we require every stage of cell growth to be hybridized three times. This type of analysis will be impossible for the Invitrogen filters. For this reason we are going to use Affymetrix gene chips for our analysis. Many factors came into play to switch to a new technology. Affymetrix chips have many distinct advantages over cDNA arrays in that they are highly reproducible and prior to a hybidization the cRNA used as probe is checked on a test chip. Wayne State University currently utilizes Affymetrix chips in the Genomics Core and there is support from the core in bioinformatics utilizing these chips. A disadvantage is cost; however, the density of the chips are much greater than cDNA arrays and this difference in cost will not affect the program.

We have prepared total RNA from all cultures and are in the process of labeling the RNA for our first hybridizations with Affymetrix chps. In contrast to cDNA arrays, the RNA used for making probe with Affymetrix chips is more rigorous and more RNA is required. Since we had only grown sufficient cells for RNA for the cDNA arrays, we had to reestablish fresh cultures of cells and to grow up large quantities of raft cultures in order to obtain sufficient RNA for the study.

**Key Research Accomplishments:**

- Have demonstrated that the cDNA microarray platform originally planned for the analysis of HOSE cell gene expression is not reliable.
- Have chosen a proven gene expression analysis platform (Affymetrix).
- Have isolated total RNA from three cultures each of two HOSE lines that exhibit the full phenotypic expression of benign to malignant cells.

**Reportable Outcomes:** None

**Conclusions:** For the first 12 months of the award, we have determined the inadequacy of the original methodology to monitor gene expression in our ovarian carcinogenesis model and have taken the appropriate steps to utilize a more suitable platform for the analysis. We have grown cells in sufficient quantity for extraction of RNA to complete the first phase of the study. The lack of description of a "premalignant" ovarian surface epithelial cell phenotype in situ has hindered progress in early diagnosis of epithelial ovarian cancer. The cell culture system we have developed mimics a premalignant condition in that there is local invasion of matrix by early passage cells. These cultures may be of value in identifying early changes in biochemical pathways that become dysregulated early during malignant progression.

**References:** None

**Appendices:** None